Apparent digestibility of selected feed ingredients in diets formulated for the sub-adult mud crab, *Scylla paramamosain* in Vietnam

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Abstract

The present study was conducted to explore the potential to incorporate local plant-based feed ingredients into diets formulated for the mud crab species, *Scylla paramamosain* commonly exploited for aquaculture in Southeast Asia. Four test ingredients (defatted soybean meal, rice bran, cassava meal and corn flour) were incorporated at 30% or 45% inclusion levels in a fishmeal-based reference diet, and used in digestibility trials where apparent digestibility coefficients (ADCs) for experimental diets and test ingredients were determined. Generally, high ADC values were obtained using diets containing 30% soybean meal or rice bran. By contrast, the lowest ADC values were obtained for the diet containing 45% cassava meal (70.9% ADMD; 77.1% ACPD; 80.2% AGED). Similar trends were observed when ADC ingredient (I) digestibilities were compared. Specifically, the highest ADCI values were obtained for soybean meal when used at a 30% inclusion level (87.6% ADMDI; 98.4% ACPDI; 95.6% AGEDI) while the lowest ADCI values were obtained using cassava meal at a 45% inclusion level (53.8% ADMDI; 60.2% ACPDI; 67.3% AGEDI). Based on the current findings we propose that soybean meal and rice bran could be considered for incorporation into formulated diets for *S.paramamosain*.

Introduction

Mud crabs of the genus *Scylla* are commercially important in several Indo-Pacific countries and they provide an important source of income and fresh food for

many coastal fishing communities (Keenan 1999). Currently, mud crab farming is well established throughout Southeast Asia with most mud crab farmers using trash fish, bivalve meats or animal by-products as feeds. This traditional feeding practice, however, is now considered unsustainable and the development of formulated low cost grow out diets is widely viewed as a priority issue for mud crab aquaculture (William & Abdullah 1999; Edwards, Tuan & Allan 2004; Christensen, Macintosh & Phuong 2004; Fielder 2004; Tuan, Anderson, Luong-van, Shelley & Allan 2006).

Consideration of any feed ingredient for incorporation into aquafeeds requires data on the target species capacity to digest and absorb it. Several recent studies have confirmed that the mud crab species *Scylla serrata* has a significant capacity to utilise feed ingredients from a variety of terrestrial animal or plant-based sources. In particular, many plant-based ingredients have been evaluated for their potential to be incorporated into aquafeeds for this species. For example, Catacutan, Uusebio & Teshima (2003) demonstrated that soybean meal, corn meal and copra meal were all highly digestible in diets formulated for *S. serrata*. Tuan *et al.* (2006) also reported apparent digestibility values for soybean meal in diets formulated for *S. serrata* which were not significantly different to those obtained using fishmeal. Likewise, Truong, Anderson, Mather, Paterson & Richardson (2008a) reported high apparent digestibility coefficients for soybean, canola, lupin and cottonseed meals incorporated into diets formulated for this species. In addition, the study of Truong *et al.* (2008a) demonstrated that the inclusion of wheat, corn or rice starch did not significantly reduce the apparent protein and energy digestibility values of formulated *S.serrata* diets.

The present study was carried out to determine the capacity of the mud crab species commonly cultured in Vietnam, *Scylla paramamosain*, to digest formulated diets containing selected, locally available plant-based ingredients (defatted soybean meal, rice bran, cassava meal and corn flour). Apparent digestibility coefficients for dry matter, crude protein and gross energy were determined for test ingredients and diets containing test ingredients. The effect of varying the inclusion level of test ingredients on nutrient digestibilities was also examined.

Materials and Methods

Experimental site and animals

The experiment was carried out at Research Institute for Aquaculture No 3 (RIA3), Nha Trang, Vietnam from 20 November 2006 to 4 January 2007 with hatchery reared sub-adult mud crabs (94.1± 1.1g) collected from a pond at RIA3. Crabs were fed a commercial diet (Turbo, C-P Feeds, Thailand) twice daily at a feeding rate of 3% body weight for a week to acclimate to experimental conditions.

Culture system

Crabs were assigned randomly into nine groups with twelve crabs in each group and held in black individual containers (19cm x 28cm x 21cm) which were covered by plastic net lids. For all experimental treatments, crabs were supplied with recirculated, aerated seawater. During the experimental period, temperature, salinity, pH, and dissolved oxygen of water were maintained at 27.5 ± 0.5^{o}C, 27.5 ± 1.5g L^{-1}, 7.67±0.09 and 4.26±0.18mg L^{-1}, respectively.

Diet preparation

Diets used in the experiment were prepared by thoroughly mixing dry ingredients, followed by wet ingredients, until a crumbly dough consistency was achieved. All diets contained 0.5% Chromic oxide (Cr_2O_3) as an inert indicator to allow the calculation of digestibility coefficients (ADC) for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED). Pilot studies determined that there was no significant loss of detectable chromium (Cr) in feed pellets immersed in water at 26^{o}C for 1h (data not shown).

94 Diet mixture was pressure pelleted using an electronic mincer with a 3mm die.
95 Pellets were steamed in a rice steamer in a microwave oven (LG) for 5 min, prior to
96 drying overnight at 50 °C in a drying oven. After drying, diet strands were cut into 8-
97 10mm strands. All experimental diets were stored at -20°C until required.

98 To ensure dietary protein levels in all test diets were set above those reported to
99 promote good growth rates in culture (Catacutan 2002), a reference diet (RF) based on
100 high quality South American fishmeal was formulated (Table 1). Eight other diets were
101 also formulated where fishmeal in the reference diet was replaced with different amounts
102 (30% and 45%) of rice bran, cassava meal, corn flour or defatted soybean meal.

103 *Feeding and faecal collection*

104 Crabs were fed experimental diets twice daily at a feeding rate of 3% body weight
105 (BW) per day until approximately 1.5 to 2g of faecal material (dry weight) was collected.
106 A daily record was kept of mortalities in each test group. Faecal material at the bottom of
107 the tank was collected by syphoning into a plastic sieve, then rinsed gently for one minute
108 in distilled water and removed individually using forceps. To collect sufficient material
109 for analysis, faecal matter from three crabs in each treatment was pooled (n=4 /
110 treatment). All samples were lyophilized and stored at -20°C until required for analysis.

111 *Chemical analysis and calculations*

112 The proximate nutrient content of experimental diets is shown in Table 2. Proximate
113 composition of diets and faecal material were determined at Nha Trang Fisheries
114 University, Vietnam, following AOAC standards (1984). Cr content of diets and faecal

115 material used in calculating apparent digestibility values were determined using the
116 method described by Furukawa & Tsukahara (1966). Apparent dry matter (ADMD),
117 crude protein (ACPD) and gross energy (AGED) digestibilities were calculated using
118 equations described by Jones & De Silva (1998):

119 ADMD = 100 – 100 (%Cr_2O_3 in feed / % Cr_2O_3 in faeces).

120 Digestibilities of crude protein (ACPD) or gross energy (AGED) were determined
121 using the formula:

122 APD = 100 – 100 [(%Cr_2O_3 in feed / % Cr_2O_3 in faeces) x (% protein or MJ kg^{-1}
123 energy in faeces/ % protein or MJ kg^{-1} energy in feed)].

124 Apparent digestibility coefficients (ADC) of test ingredients were calculated using
125 equations described by Bureau, Harris & Cho (1999).

126 $ADC_I = ADC_T + ((1 - s)\ D_R / s\ D_I)\ (ADC_T - ADC_R)$; where: ADC_I = apparent
127 digestibility coefficient of test ingredient; ADC_T = apparent digestibility
128 coefficient of test diet; ADC_R = apparent digestibility coefficient of the reference
129 diet; D_R = % nutrient (or kJ/g gross energy) of the reference diet mash; D_I = %
130 nutrient (or kJ/g gross energy) of the test ingredient; *s* = proportion of test
131 ingredient in test diet mash (i.e. 0.3 and 0.45 in this study); (1 – *s*) = proportion of
132 reference diet mash in test diet mash (i.e. 0.7 and 0.55 in this study).

133 *Statistical analyses*

134 The significance of data were determined by one- way ANOVA (SPSS version
135 13.0) and *post hoc* comparison by Tukey's HSD. For all analysis the significance level
136 of $p<0.05$ was used as standard.

Results

Digestibility determinations: Experimental diets

Apparent digestibility coefficients for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) obtained using experimental diets are presented in Table 3. ADMD coefficients ranged from 70.9% to 85.7%. The highest ADMD value was obtained using the diet containing 30% soybean meal (SBM30) which was significantly higher ($p<0.05$) than those obtained using any other diet except that containing 30% rice bran (RB30) or the reference (RF) diet. By contrast, the lowest ADMD value was obtained using the diet containing 45% cassava meal (CM45) which was significantly less ($p<0.05$) than those obtained for all other experimental diets. It was also shown that incorporation of more than 30% soybean or cassava meal into experimental diets resulted in a significant reduction ($p<0.05$) in ADMD values.

ACPD coefficients obtained using experimental diets ranged from 77.1% to 93.2%. ACPD coefficients for diets containing soybean meal (SBM30, SBM45) or 30% rice bran (RB30) were either significantly higher ($p<0.05$) or equivalent to those obtained using the fishmeal-based reference (RF) diet. By contrast, the lowest ACPD value was obtained using the diet containing 45% cassava meal (CM45) which was significantly lower ($p<0.05$) than those obtained using any other experimental diet. Furthermore, it was demonstrated that if the level of any test ingredient incorporated into experimental diets was increased from 30% to 45%, there was a significant reduction ($p<0.05$) in the ACPD value of the diet.

AGED coefficients for experimental diets were generally high and ranged from 80.2% to 92.2%. The highest AGED value was obtained using the diet containing 30% soybean meal (SBM30) which was significantly higher ($p<0.05$) than those obtained using other experimental diets except the diet containing 30% rice bran (RB30) or the reference (RF) diet. By contrast, the lowest ACPD value was obtained using the diet containing 45% cassava meal (CM45) which was significantly lower ($p<0.05$) than that obtained from other experimental diets. It was also demonstrated that if the level of rice bran, cassava meal or soybean meal incorporated into diets was increased from 30% to 45% there was a significant reduction ($p<0.05$) in the AGED value of the diet.

Digestibility determinations: Test ingredients

Apparent dry matter (ADMDI), crude protein (ACPDI) and gross energy (AGEDI) digestibility coefficients calculated for specific feed ingredients are presented in Table 4. The highest ADMDI value (87.6%) was obtained for soybean meal (at 30% inclusion level) which was significantly higher ($p<0.05$) than values obtained for all other test ingredients. By contrast, ADMDI values obtained for cassava meal were significantly lower than those obtained for all other test ingredients. ACPDI values were obtained for soybean meal (at any inclusion level) and rice bran (at a 30% inclusion level) that were significantly higher ($p<0.05$) than those obtained for any other test ingredient. . By contrast, the ACPDI value obtained for cassava meal (at 45% inclusion level) was significantly lower than those obtained for all other test ingredients. The ingredient with the highest AGEDI value (95.6%) was soybean meal, when used at a 30% inclusion level,

while values obtained using cassava meal were significantly lower ($p<0.05$) than those obtained for any other test ingredient.

Discussion

In a previous study, we demonstrated that *S. serrata* has a high capacity to digest a range of plant based feed ingredients (Truong *et al*. 2008a). In the present study we have extended these investigations and examined the potential to incorporate local plant based feed ingredients into diets formulated for the mud crab species commonly cultured in Southeast Asia (*S.paramamosain).* In agreement with our previous finding on *S. serrata, S.paramamosain* demonstrated a high capacity to digest soybean meal at inclusion levels up to 45%. These results are consistent with the findings of Catacutan *et al*. (2003) who reported that ADC values for dry matter and crude protein in diets formulated for *S.serrata* were 90.9% an 95.5%, respectively. Likewise, Tuan *et al.* (2006) reported that ADC coefficients for dry matter, energy and crude protein for soybean meal were relatively high with values of 95.7%, 97.1% and 97.9% respectively.

In this study, at a 30% inclusion level rice bran demonstrated generally high ADC values. This finding is in close agreement with the observations of Catacutan *et al*. (2003) who demonstrated that dry matter and crude protein digestibility coefficients for rice bran in *S.serrata* were relatively high (89% and 94% respectively). In a related study, Truong *et al.* (2008a) also reported high ADC coefficients for rice-based ingredients incorporated into diets formulated for *S.serrata.* Furthermore, incorporation of rice starch into fishmeal-based diets formulated for juvenile *S.serrata* did not appear to significantly reduce growth performance (Truong, Anderson, Mather, Paterson & Richardson. 2008b). Based on the above findings, and those of the current investigation, we suggest that rice-based products, such as bran and starch, are generally well digested and should be

investigated further for their potential to be incorporated into aquafeeds formulated for *Scylla* species.

A key finding of this study was that several of the ADC values for corn flour were significantly less than those obtained for rice bran or soybean meal. This result is surprising considering that Truong *et al.* (2008a) reported that *S.serrata* demonstrated a high capacity to digest diets containing corn starch. Likewise, Catacutan *et al.* (2003) reported high ACPD (96.4%) and ADMD (93.2%) coefficient values for *S.serrata* diets containing 30% corn flour. A possible explanation for these apparent discrepancies is that there may be significant differences in the capacity of the various *Scylla* species to digest corn-based ingredients. Alternatively, the capacity of mud crabs to digest corn-based ingredients may be influenced by the source or preparation of the corn-based ingredient.

Relatively poor ADC values were obtained using diets containing cassava meal. This result is in contrast to other studies which have examined the potential of cassava meal for incorporation into crustacean aquafeeds. For example, Gomes & Pena (1997) reported that inclusion of 30% heated cassava meal in diets formulated for *Macrobrachium rosenbergii* did not significantly reduce digestibility coefficients for protein and energy. The relatively poor digestibility of cassava meal in diets formulated for mud crabs might be a consequence of the presence of toxic factors in this ingredient. Specifically, Oboh & Akindahunsi (2005) reported that rats fed a diet containing 40% cassava meal had a significant rise in the serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase activity indicating possible damage to the liver and/or heart. Further studies will be required to determine what factors may be

227 inhibiting the digestion of cassava meal in *S.paramamosain* and if the relatively poor
228 digestibility of this ingredient is consistent across other *Scylla* species.

229 An important observation of the current study was that several ADC values for
230 diets and test ingredients were significantly reduced when ingredient inclusion levels
231 were raised. For example, the ACPDI values for rice bran and cassava meal were reduced
232 by approximately 9% and 15%, respectively, when the dietary inclusion level was raised
233 from 30% to 45%. These findings are consistent with those of other workers who have
234 reported increased incorporation of plant-based materials can impact negatively on the
235 digestibility of aquaculture diets. For example, Hansen, Rosenlund, Karlsen, Olsvik &
236 Hemre (2006) reported that plant-based ingredients reduced protein and fat digestibility
237 in formulated cod diets. Likewise, alpha-cellulose reduced the digestibility of diets
238 formulated for the shrimp *Macrobrachium rosenbergii* (Gonzalez-Pena, Anderson, Smith
239 & Moreira 2002). By contrast, other workers have demonstrated that increased
240 incorporation of plant-based materials can improve aquaculture diet digestibility. For
241 example, Bautista-Teruel, Eusebio & Welsh (2003) reported that higher ADC values for
242 dry matter and crude protein were achieved if the level of feed pea meal incorporated into
243 practical diets for *Penaeus monodon* was increased. Likewise, cornstarch has been used
244 to improve the digestibility of diets formulated for the white shrimp, *Litopenaeus*
245 *vannamei* (Guo, Liu, Tian & Huang 2006).

246 At present, it is unclear why there are differences in the impacts of plant-based
247 ingredients on aquaculture diets. Such variations may reflect species-related differences
248 in the capacity of candidate organisms to digest plant-based materials. For example, in

these laboratories it has been shown that enzymes, such as cellulase, which are required for the breakdown of plant-based materials are present at much higher levels in the digestive system of the omnivorous redclaw crayfish *Cherax quadricarinatus*, than in digestive tissues from the carnivorous mud crab *S.serrata* (Pavasovic, Richardson, Anderson, Mann & Mather 2004; Pavasovic, Anderson, Mather. & Richardson 2007). It is also possible that differences in the digestibility of plant-based materials may reflect how the addition of these materials has affected the levels of other dietary components. For example, Guo *et al* (2006) speculated that adding corn starch to shrimp diets helped improve digestibility by permitting a reduction in the level of less digestible dietary components, such as cellulose.

In conclusion, the current investigation has shown that at a 30% inclusion level, soybean meal and rice bran did not impact negatively on the digestibility of fishmeal-based artificial diets. Moreover, even at an inclusion level of 45%, crude protein and gross energy digestibility of experimental diets was not significantly reduced by the presence of soybean meal. This suggests that soybean meal and rice bran are ingredients with high potential for inclusion into diets formulated for *S. paramamosain*. By contrast, corn flour demonstrated only limited potential for inclusion into diets formulated for *S.paramamosain*. In particular ADC values were generally less than those observed in similar studies performed using *S.serrata*. Another important finding of the current study was that cassava meal demonstrated poor potential for inclusion in diets formulated for *S.paramamosain*, at least over the inclusion range tested.

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375 Table 1: Composition of diets formulated for the digestibility trial using different local

376 ingredients (% dry weight basis).

Ingredient (%)	Diet								
$g100g^{-1}$	RF[1]	RB30	RB45	CM30	CM45	CF30	CF45	SBM30	SBM45
Fish meal[2]	81.5	51.5	36.5	51.5	36.5	51.5	36.5	51.5	36.5
Binder (Wheat gluten)	5	5	5	5	5	5	5	5	5
Cod liver oil[3]	3	3	3	3	3	3	3	3	3
CaHPO4	3	3	3	3	3	3	3	3	3
Common Ingredients[4]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Rice bran (RB)[5]	-	30	45	-	-	-	-	-	-
Cassava meal (CM)[5]	-	-	-	30	45	-	-	-	-
Corn flour (CF)[5]	-	-	-	-	-	30	45	-	-
Soybean meal (SBM)[5]	-	-	-	-	-	-	-	30	45

377 [1] Reference diet

378 [2] Peruvian fish meal

379 [3] Healthier of Australia (Healtheries of New Zealand, Auckland, New Zealand)

380 [4] Common ingredients ($g100g^{-1}$): dried squid (5%); chromic oxide (Cr_2O_3) (0.5%);

381 mineral and vitamin premix (2%), (active ingredients per kg^{-1} of premix 4.68 g

382 K_2HPO_4; 7.12 g $MgSO_4.7H_2O$; 1.84 g $NaH_2PO_4.2H_2O$; vitamin premix (kg^{-1}) - 100000

383 IU vitamin retinol; 500 mg thiamine; 1.75g riboflavin; 1.125g 1125 mg pyridoxine

384 hydrochloride; 3.75g cyanocobalamin; 25g ascorbic acid; 50g colecalciferol; 20 000 IU

385 d-alpha-tocopheryl acid succinate; 50 mg biotin).

386 [5] Products of Vietnam

Table 2: Dry matter (DM), gross energy (GE MJ kg^{-1}), crude fat (CF), crude protein (CP) and ash of experimental diets and test ingredients used in the formulated diets (% dry weight basis)

Sources	DM (%)	CP (%)	CF (%)	Ash (%)	GE[1] (MJ kg^{-1})
Experimental diets					
RF	93.6	65.3	11.2	16.4	20.4
RB30	91.2	48.5	11.7	11.7	18.9
RB45	90.1	40.1	11.4	9.3	18.6
CM30	91.1	46.5	10.8	11.7	18.6
CM45	92.4	37.1	10.0	9.3	18.0
CF30	89.0	46.6	11.2	14.4	18.8
CF45	90.9	37.2	10.6	13.3	18.4
SBM30	93.4	56.0	10.2	13.8	19.6
SBM45	93.8	51.4	9.4	12.4	19.6
Test ingredients					
Fish meal	93.6	71.2	8.7	16.8	21.0
Defatted soy-bean	91.2	40.1	1.4	7.4	20.1
Rice bran	90.2	15.1	6.8	8.1	18.8
Cassava meal	93.6	8.2	3.6	9.2	17.4
Corn flour	92.2	8.6	4.9	4.6	18.2

[1] Determined using a bomb calorimeter at Nha Trang University

392 Table 3: Apparent digestibilities (%) for dry matter (ADMD), crude protein (ACPD) and

393 gross energy (AGED) in experimental diets

Diet	ADMD	ACPD	AGED
RF	84.9 ± 0.5^{e}	90.9 ± 0.5^{d}	90.7 ± 0.7^{de}
RB30	82.6 ± 0.2^{de}	91.8 ± 0.4^{d}	90.6 ± 0.4^{de}
RB45	80.6 ± 0.4^{cd}	87.8 ± 0.4^{c}	87.3 ± 0.7^{c}
CM30	75.8 ± 0.9^{b}	84.7 ± 0.8^{b}	83.8 ± 1.1^{b}
CM45	70.9 ± 0.6^{a}	77.1 ± 0.7^{a}	80.2 ± 0.5^{a}
CF30	80.2 ± 0.1^{cd}	87.8 ± 0.2^{c}	88.7 ± 0.5^{cd}
CF45	78.9 ± 0.3^{c}	83.6 ± 0.3^{b}	85.6 ± 0.37^{bc}
SBM30	85.7 ± 0.3^{e}	93.2 ± 0.4^{e}	92.2 ± 0.4^{e}
SBM45	81.6 ± 0.2^{cd}	92.1 ± 0.9^{d}	88.6 ± 0.3^{cd}

394 Values are means ± SE (n = 4 replicates per treatment). Means in the same column with

395 the same superscript are not significantly different ($p>0.05$) from one another

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403 Table 4: Apparent digestibility coefficients (%) for dry matter (ADMDI), crude protein

404 (ACPDI) and gross energy (AGEDI) of test ingredients used in formulated diets

Sources	Inclusion (%)	ADMDI (%)	ACPDI (%)	AGEDI (%)
Rice bran	30	77.3 ± 1.7^{c}	93.8 ± 0.4^{d}	90.2 ± 1.8^{cd}
	45	75.3 ± 1.2^{bc}	84.0 ± 0.7^{c}	83.1 ± 1.6^{bc}
Cassava meal	30	54.8 ± 0.9^{a}	70.3 ± 2.6^{b}	67.8 ± 0.9^{a}
	45	53.8 ± 1.1^{a}	60.2 ± 1.3^{a}	67.3 ± 1.1^{a}
Corn flour	30	69.2 ± 1.2^{b}	80.6 ± 0.9^{c}	83.9 ± 1.9^{bc}
	45	71.5 ± 0.8^{bc}	74.7 ± 0.5^{b}	79.4 ± 1.4^{b}
Defatted soy bean	30	87.6 ± 2.4^{d}	98.4 ± 0.4^{d}	95.6 ± 2.9^{d}
	45	77.5 ± 0.5^{c}	93.5 ± 0.2^{d}	86.1 ± 0.7^{bc}

405 Values are means ± SE (n = 4 replicates per treatment). Means in the same column with

406 the same superscript are not significantly different ($p>0.05$) from one another

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